Journal of Advances in Biology & Biotechnology

24(2): 24-33, 2021; Article no.JABB.67117
ISSN: 2394-1081

Viability and *in vivo* Hypocholesterolemic Effect of *Lactobacillus plantarum* 29V in Local Honey

Ulrich Landry Kamdem Bemmo[1,2*], Chancel Hector Momo Kenfack[1], Jean Marcel Bindzi[2], Raoul Borkeum Barry[2] and François Zambou Ngoufack[1]

[1]Research Unit of Biochemistry, Food Science and Nutrition (URBPMAN), Department of Biochemistry, Faculty of Science, University of Dschang, P.O.Box 67, Dschang, Cameroon.
[2]Department of Life Sciences, Higher Teacher Training College of Bertoua, University of Ngaoundere, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Author ULKB designed the study, wrote the protocol, performed the experimental analyses and wrote the first draft of the manuscript. Author CHMK participated in lab works and analyzing the data. Authors JMB and RBB contributed to the review of the manuscript. Author FZN supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2021/v24i230199
Editor(s):
(1) Dr. Evangel Kummari, Texas A&M University, USA.
Reviewers:
(1) Bernadette E. Teleky, University of Agricultural Sciences and Veterinary Medicine; Cluj-Napoca, Romania.
(2) Gheorghe-Adrian Martau, University of Agricultural Sciences and Veterinary Medicine, Romania.
(3) Terd Disayathanoowat, Chiang Mai University, Thailand.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67117

Original Research Article

Received 06 February 2021
Accepted 13 April 2021
Published 23 April 2021

ABSTRACT

The conservation of probiotic products requires low temperatures and suitable equipment that are less available in developing countries. The challenge today is to find a local food matrix that can also carry probiotics (microorganisms with benefits for consumers) in the gastro-intestinal tract. The study mainly focus in the current research was to study the use of honey as a food matrix to carry probiotics in treating the cardiovascular disease, hypercholestreolemia. Thus, this study aimed to assess the viability of *Lactobacillus plantarum* 29V and its *in vivo* hypocholesterolemic properties when contained in honey. The strain *L. plantarum* 29V was added in pasteurized honey and was studied its viability in honey and its impact on the physicochemical parameters of honey.
For *in vivo* studies, 0.5 mL of the pasteurized honey containing approximately 10^8 CFU/mL of *L. plantarum* 29V were administered to rats fed on a cholesterol-enriched diet (control diet+ pure

**Corresponding author: E-mail: ulrichbemmo@yahoo.com;*

cholesterol solution (0.04 g/mL) per day per rat) using a feeding syringe; the treatment lasted 4 weeks. Serum lipids were analyzed during the experiment. The results have shown that the probiotic strain *L. plantarum* 29V can survive in honey for 28 days without affecting the honey's qualities. Even present in honey, this strain continues to lower serum total cholesterol, (VLDL +LDL)-cholesterol and triglycerides levels of hypercholesterolemic rats. In addition, HDL-cholesterol levels significantly increased, and the atherosclerosis index was significantly lowered. The present study revealed that honey could be used as a food matrix to carry the probiotic *Lactobacillus plantarum* 29V strain very well into the gastro-intestinal tract. Hence, a probiotic formulation made of pasteurized honey and *L. plantarum* 29V would be used to treat or prevent hypercholesterolemia if these effects are confirmed in Human beings.

Keywords: Viability; Lactobacillus plantarum 29V; honey; and hypocholesterolemia.

1. INTRODUCTION

Cholesterol is a lipophilic molecule that is essential for human life. It has many roles that contribute to normally functioning cells. For example, cholesterol is an important component of the cell membrane. It contributes to the structural makeup of the membrane as well as modulates its fluidity. Cholesterol functions as a precursor molecule in the synthesis of vitamin D, steroid hormones, and sex hormones [1].

Cholesterol plays a detrimental role in the pathogenesis of atherosclerosis and cardiovascular diseases (CVDs) [2]. CVDs are the major cause of death globally, taking an estimated 17.9 million lives each year. [3]. Hence, the WHO has predicted that by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the World [4]. Medicinal treatments exist (fibrates, inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A reductase); but they are expensive and have side effects [5]. A safe alternative approach to fight against CVDs is the use of probiotics (beneficial live microorganisms) such as lactic acid bacteria (LAB) with active bile salt hydrolase (BSH). These microorganisms with active BSH can be conveyed in the gastrointestinal tract (GIT) via food matrices in order to lower cholesterol levels through interaction with host bile salt metabolism [6]. Fermented dairy products such as yogurt, cheese, and infantile formulae milky, constitute one of the most effective means of conveying probiotics lactic bacteria into the gastrointestinal tract. However, these products are not easily accessible by the rural populations of developing countries and require specific equipment for their conservation. Thus, it would be judicious to find local foods able to convey the probiotics, easily accessible by everybody and easy to preserve in our environment. The strain *Lactobacillus plantarum* 29V was isolated from raw milk in the locality of Fongo-Tongo in West-Cameroon [7]. Moreover, Sieladie in 2012 showed that this strain of *Lactobacillus plantarum* 29V has an interesting hypocholesterolemic effect in *Wistar* rats when it is administered by oral force-feeding. However, the ability of this strain to lower cholesterol levels when it is carried in GIT by a food matrix has not been worked out as yet [8]. Prebiotics are substances which cannot be digested by pathogenic microorganisms and thereby selectively enhance the development of probiotics. Honey being an exceptional source of such prebiotic oligosaccharides (fructo- and galacto- oligosaccharides) could be used to produce non-dairy probiotics formulations. Moreover, honey is a natural food consumed by humans since many centuries by almost all civilizations due to its rich nutritional and therapeutic values. It remains stable at room temperature for long time [9]. Hence, this present work consists of studying the hypocholesterolemic effect of *L. plantarum* 29V strain contained in honey. To achieve this objective, *L. plantarum* 29V was added firstly in the pasteurized honey and its viability in honey was studied. The impact of the strain on the physicochemical parameters of honey was also evaluated. After that, were assessed the effects of the ingestion of a formulation made of pasteurized honey and *L. plantarum* 29V on the lipidic profile of *Wistar* rats fed on a cholesterol-enriched diet.

2. MATERIAL AND METHODS

2.1 Microorganism

The strain *Lactobacillus plantarum* 29V used in the study was obtained from the stock culture collection of our Research Unit of Biochemistry, Food Science and Nutrition (RUBPMAN) of the University of Dschang. This strain was isolated from raw cow milk in the western highlands of

Cameroon and identified as *Lactobacillus plantarum* based on physiological and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l'Etoile France) and rep-PCR genomic fingerprinting [7]. Lactobacillus plantarum 29V strain was maintained as frozen stocks at - 20 °C in MRS broth (Biolife®, Milano, Italy) supplemented with glycerol (30% v/v) and propagated twice (1% inoculum) in MRS broth for 14 – 16 h at 37 °C before experimental use.

2.3 Assessment of the Viability of *Lactobacillus plantarum* 29V in Honey

2.3.1 Preparation of Honey Samples, Inoculation, and Storage

In this study, was used a wild polyfloral honey sample, procured from a local beekeeper of the sudano-guinean zone of West-Cameroon. It was obtained by draining the honey after manually uncapping the comb frames, and pasteurized at 63 °C for 30 min.

The growth of *Lactobacillus plantarum* culture in MRS broth was monitored using a spectrophotometer (UNICO UV-2000 UV/Vis Spectrophotometer). At the early stationary growth phase, bacterial cells were harvested by centrifugation (4,000×g, 10 min, 4 °C). The pellet was then washed twice with 0.01 mol/l potassium phosphate buffer with pH 7.0. The cells' pellet was suspended in 100 mL sterile potassium phosphate buffer 0.01 mol/L and stored at − 20 °C until used. The viable cell count of this suspension was determined by enumeration on MRSCys-HCl agar plates at 37 °C for 48 h. 120 µL of this suspension (~ 2× 10^8 CFU/mL) was introduced into 100 mL of honey and thoroughly homogenized. Noninoculated honey samples were used as the control. Inoculated and non-inoculated honey samples were stored at 25 °C (room temperature) and 4 °C for 28 days.

2.3.2 Enumeration of Cell Viability of the Strains in Honey

From the inoculated honey samples, an aliquot of 10 mL was taken after every 7 days for the viability assessment. For this, tenfold serial dilutions were done in 0.1% peptone water while homogenizing by vortexing, followed by viable cells count using MRS agar. The plates were incubated anaerobically at 37 °C for 48 h.

2.3.3 Honey Samples and Physicochemical Analyses

Some physicochemical characteristics of honey samples were evaluated. The moisture was determined using the IUPAC method [10]. The pH was measured by pH meter in a solution containing 1 g of honey sample in 20 mL of distilled water according to the Association of Official Analytical Chemists [11]. For the determination of free acidity, the harmonized method of the International Honey Commission was used [12]. The total carbohydrate content of honey samples was estimated by the phenol sulfuric acid method at 480 nm. Reducing sugars in honey samples were estimated by Dinitro Salicylic Acid (DNSA) method at 530 nm.

2.4 Experimental Animals

A total of thirty-six (36) adult male *Wistar* rats (mean body weight 160-165 g) were procured from the animal house of the Department of Biochemistry at the University of Dschang where the room temperature was 22-25 °C. The animals were divided into six homogeneous groups comprising six animals each one and maintained under a constant 12 h light–12 h dark cycle.

The basic composition of the experimental diet is shown in Table 1. Animal diets were formulated according to Telefo [13].

Table 1. Food composition in g/100 g of flour

Constituents	g/100g
Corn flour	67.8
Soya beans flour	20
Fish flour	10
Bone powder	1
Kitchen salt	1
Groundnut oil	0.1
Multi vitamins complex	0.1

2.5 Feeding Schedule

Rats were acclimatized for one week to remove the effect of stress experienced by the animals due to separation from the main stock and to become accustomed to the testing diet. At the end of the adaptation period, all the groups were fed on their assigned experimental diets for the next four weeks. During the entire period of experimentation, the rats had free access to water and control diet. Body weight was measured weekly.

Table 2. Experimental Groups and feed rations received per day

Groupes	Traitements
CD	Control diet
ChCD	Pure cholesterol solution (0.04 g/mL) using feeding syringe + Control diet
29VCD	0.5 mL of *Lbp* 29V (approximately 10^8 ufc/mL using feeding syringe + Control diet
ChFCD	Pure cholesterol solution (0.04 g/mL) + 0.5 mL of probiotic formulation (honey solution 100 % (v/v) containing approximately 10^8 CFU/mL of *L. plantarum* 29V) using feeding syringe + Control diet
ChHCD	Pure cholesterol solution (0.04 g/mL) + 0.5 mL of honey using feeding syringe + Control diet
ChACD	Pure cholesterol solution (0.04 g/mL) + atorvastatin (10 mg/Kg) using feeding syringe + Control diet

2.6 Experimental Design

This table provides a detailed formula of experimental diets used in the present study.

2.7 Blood Sampling and Analytical Procedures

After every two weeks of the experiment, three animals of each group were randomly drawn and anesthetized with chloroform. The samples of blood were collected by cardiac puncture after incision of the abdominal cavity of the rats. Each blood sample was centrifuged at 3000 rpm for 15 minutes (Labofuge A, Heraeus, Germany). The resulting serum was used to analyze total cholesterol, TAG and HDL-cholesterol using commercial enzymatic kits (INMESCO). The fractions VLDL + LDL were determined according to Trinder's equation [14]:

Cholesterol-(VLDL+LDL) = Cholesterol-total – Cholesterol-HDL

Atherosclerosis index was also determined by using the following relation:

Atherosclerosis index = Cholesterol-(VLDL + LDL) / Cholesterol-HDL

2.8 Statistical Analysis

Data are representative of the average, and standard deviation of two or three tests carried out independently; these results were analyzed by the test of Variance Analysis (ANOVA) using the software GraphPad InStat (GraphPad Software Inc, V3), followed by a comparison of the means between them by the test of Student-Newmann-Keuls to the threshold of probability 5%.

3. RESULTS AND DISCUSSION

3.1 Viability of *L. plantarum* 29V in Pasteurized Honey and Physico-chemical Parameters of Honey

The viability rate of the strain *L. plantarum* 29V decreased significantly in the pasteurized honey samples stored at room temperature from day 21. During storage, the pH of the various honey samples varied between 4.24 and 4.33; free acidity ranged from 98.00 mEq/Kg to 101.00 mEq/Kg and density varied between 1.31 and 1.38 g/mL. However, no significant difference ($P<0.05$) was observed between its values for each parameter. Table 3 shows the evolution of the pH, free acidity, density and viable cells count of *L. plantarum* in honey samples stored at different conditions.

The strain *L. plantarum* 29V remains viable in honey after 28 days of storage at 4 °C and 25 °C. However, better viability was observed in honey stored at 4 °C compared to honey stored at 25 °C. These results are closer to those of Bemmo et al. [15] and Zambou et al. [16], who respectively showed that honey inoculated with *Lactobacillus plantarum* GLP56 and *Lactobacillus plantarum* 2S strains showed better viability in honey stored at 4 °C than honey stored at 25 °C after 28 days.

The viability of *L plantarum* 29V in honey after 28 days of conservation could also be allotted to its ability to resist at pH lower than 4 [7] and due to the presence of oligosaccharides (fructo- and gluco- oligosaccharides) in honey recognized as prebiotics. It is well known that prebiotics stimulate the growth and ensure the viability of lactic acid bacteria [17,18]. The studies of Bemmo et al. [15] demonstrated that *Lactobacillus plantarum* GLP56,

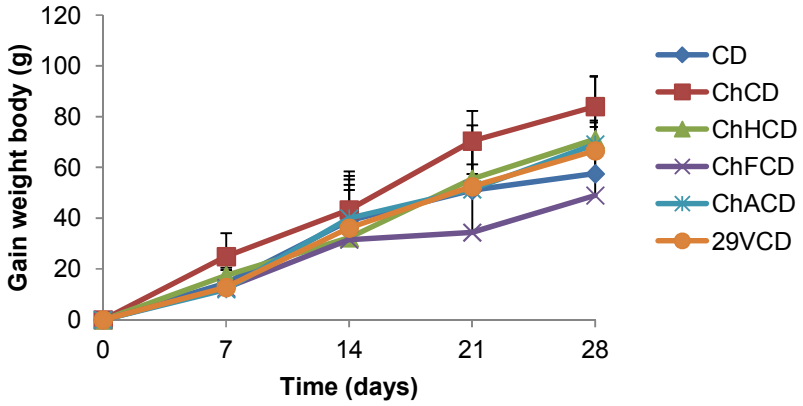

Fig. 1. Gain weight body

CD : Rats fed Control diet
ChCD : Rats fed pure cholesterol solution (0.04 g/mL) + Control diet
ChHCD : Rats fed pure cholesterol solution (0.04 g/mL) + 0.5 mL of honey + Control diet
ChFCD : Rats fed pure cholesterol solution (0.04 g/mL) + 0.5 mL of probiotic formulation + Control diet
ChACD : Rats fed pure cholesterol solution (0.04 g/mL) + atorvastatine (10 mg/Kg)
29VCD : Rats fed 0.5 mL of Lbp 29V (approximately 10^8 UFC/mL) + Control diet

Table 3. Viable cells count of *L. plantarum* 29V in honey samples stored at 4°C and 25°C and physicochemical characteristics of honey samples

Parameters	Duration of storage (days)	Honey + *L. plantarum* 29V stored at refrigeration temperature (4°C)	Honey + *L. plantarum* 29V stored at room temperature (25°C)	Honey stored at refrigeration temperature (4°C)	Honey stored at room temperature (25°C)
Viable cell counts of L. plantarum Log CFU/mL and % viability	0	8.49 ± 0.14 (100%)	8.49 ± 0.14 (100%)	0	0
	7	8,26 ± 0.87 (97.29%)	6.79 ± 0.73 (79.97%)	0	0
	14	7.75 ± 0.62 (91.28%)	6.86 ± 0.38 (80.80%)	0	0
	21	7.13 ± 0.28 (83.98%)	5.98 ± 0.19* (70.43%)	0	0
	28	7.20 ± 0.12 (84.80%)	5.08 ± 0.60* (59.83%)	0	0
pH	0	4.25 ± 0.00	4.25 ± 0.00	4.25 ± 0.00	4.25 ± 0.00
	7	4.24 ± 0.01	4.26 ± 0.02	4.25 ± 0.01	4.25 ± 0.01
	14	4.28 ± 0.01	4.25 ± 0.01	4.26 ± 0.03	4.25 ± 0.01
	21	4.26 ± 0.01	4.32 ± 0.01	4.27 ± 0.02	4.27 ± 0.01
	28	4.28 ± 0.02	4.32 ± 0.01	4.26 ± 0.01	4.28 ± 0.01
Free acidity	0	99.5 ± 0.49	99.5 ± 0.49	99.5 ± 0.49	99.5 ± 0.49
	7	100.1 ± 0.28	100.25 ± 0.35	99.5 ± 0.00	100 ± 0.71
	14	99.5 ± 0.71	100.55 ± 2.75	99 ± 2.12	100.55 ± 2.75
	21	99.5 ± 1.41	100 ± 0.71	99.25 ± 0.35	99.5 ± 2.12
	28	99.55 ± 1.34	99.5 ± 2.12	100 ± 0.71	100.25 ± 0.35
Density	0	1.36 ± 0.01	1.36 ± 0.01	1.36 ± 0.01	1.36 ± 0.01
	7	1.33 ± 0.01	1.32 ± 0.01	1.30 ± 0.05	1.31 ± 0.01
	14	1.31 ± 0.04	1.35 ± 0.01	1.307 ± 0.03	1.35 ± 0.01
	21	1.34 ± 0.06	1.37 ± 0.00	1.39 ± 0.01	1.37 ± 0.00
	28	1.37 ± 0.08	1.38 ± 0.01	1.34 ± 0.04	1.32 ± 0.01

Lactobacillus plantarum GLA51 and *Lactobacillus plantarum* GGU strains were able to resist acid stress due to the presence of *clpL* gene encoding for F1F0 ATPase pump involved in the resistance of bacteria against the acid stress.

The number of viable cells of *L plantarum* 29V in honey does not increase because of the low water and protein contents of honey, as the growth of lactobacilli requires a complex medium containing proteins and certain growth factors [19].

3.2 Body Weight Gain

Fig. 1 represents the results of the body weight gain of the rats. The groups ChFCD, 29VCD, ChACD, and ChHCD showed no significant differences (P>0.05) in body weight gain compared to the negative control group. This indicates that *L. plantarum* 29V, honey, and atorvastatin do not affect the growth of rats.

3.3 Lipid profiles: Total Cholesterol, HDL-Cholesterol, (VLDL+LDL)-cholesterol, Triglycerides, Atherosclerosis Index

Figure 2a shows the serum total cholesterol of rats during the experimental period. The total cholesterol level in the group ChCD is significantly higher than that of the other groups and no significant difference is observed between the cholesterol level of groups CD, ChFCD, ChHCD, 29VCD, and ChACD. Figure 2b shows the HDL-cholesterol of rats during the experimental period**.** During the first two weeks of experimentation, was observed a significant reduction (p<0.05) of HDL-Cholesterol in group ChCD while it increases significantly (p<0.05) in the groups ChACD and 29VCD. Nonetheless, this HDL-Cholesterol in groups ChACD and 29VCD is significantly higher than that of the other groups. During the two last weeks, the groups ChFCD and ChCD show a significant increase (p<0.05) of HDL-cholesterol while it significantly decreases (p<0.01) in the group 29VCD.Group ChFCD presents a significantly high level of HDL-cholesterol (p<0.05) compared to group CD. Figure 2c shows the (VLDL+LDL)-cholesterol of rats during the treatment. During the first two weeks, the cholesterol-(VLDL + LDL) increases significantly (p<0.01) in group ChCD and decreases significantly (p<0.05) in group ChACD. Thus, group ChCD shows a level of cholesterol-(VLDL + LDL) significantly higher than that of other groups while group ChACD shows cholesterol-(VLDL + LDL) significantly lower than that of the other groups. At the fourth week, cholesterol-(VLDL + LDL) decreases significantly in the groups ChFCD (p<0.001), ChCD (p<0.01), ChHCD (p<0.05), and 29VCD (p<0.05). It increases significantly in the group ChACD. However, group ChCD has a level of cholesterol-(VLDL + LDL) significantly higher than that of other groups. Cholesterol-(VLDL + LDL) is significantly lower in group ChFCD compared to that of the other groups but is comparable with that of group 29VCD. The evolution of triglycerides is shown in the figure 2d. During the first 14 days of experimentation, the triglyceride increases significantly in the groups ChCD (p<0.001) and ChHCD (p<0.05). We observe a significant reduction in the animals of groups ChFCD (p<0.05) and ChACD (p<0.001). However, in the group ChFCD it is significantly lower than that of the groups CD, ChCD, ChHCD, and 29VCD. Between the second and the fourth week, we observe a significant increase (p<0.001) of triglyceride in the group ChACD whereas this parameter decreases significantly (p<0.001) in all the other groups. The triglyceride remains significantly lower in group ChFCD compared to that of the other groups. Figure 2e shows the atherosclerosis index during the experimental period. During the first two weeks, the atherosclerosis index increases significantly (p<0.01) in the group ChCD and drops significantly (p<0.05) in the groups ChACD and A29V.However, the atherosclerosis index is significantly higher (p<0.01) in group ChCD than that of other groups. Between the second and the fourth week, the atherosclerosis index decreases significantly (p<0.01) in the groups ChCD and ChFCD; however, this parameter significantly remains lower (p<0.01) in groups ChFCD and 29VCD, compared with that of the group ChCD. The atherosclerosis index increases significantly in group ChACD.

High concentrations of total cholesterol and LDL-Cholesterol are highly associated with an increased risk of coronary heart disease; thus, reduction in total cholesterol and LDL-Cholesterol in hypercholesterolemic men can reduce the incidence of cardiovascular disease [20]. Our results showed that the administration of the formulation made of Honey and *L. plantarum* 29V strain to rats with induced hypercholesterolemia did not significantly affect the body weight. The results obtained here

correlated with the findings of Kumar et al. [21], Liong and Shah [22], and Wang et al. [23], who used *Lactobacillus plantarum* Lp21 et Lp91, *L. casei* ASCC 292, and *L. plantarum* MA2 respectively, as supplements to a high-cholesterol diet and found a little change in body weight gain.

The present study showed that administration of the formulation (pasteurized honey and *L. plantarum* 29V), honey, and atorvastatin resulted in a reduction of total serum cholesterol, (VLDL+LDL)-cholesterol and triglycerides levels of hypercholesterolemic rats. However, during all the period of treatment, the total cholesterol, (VLDL+LDL)-cholesterol and triglycerides levels remained lower (without any significant difference (p>0.05)) in the rats which were treated with the formulation (pasteurized honey and *L. plantarum* 29V) compared to those treated

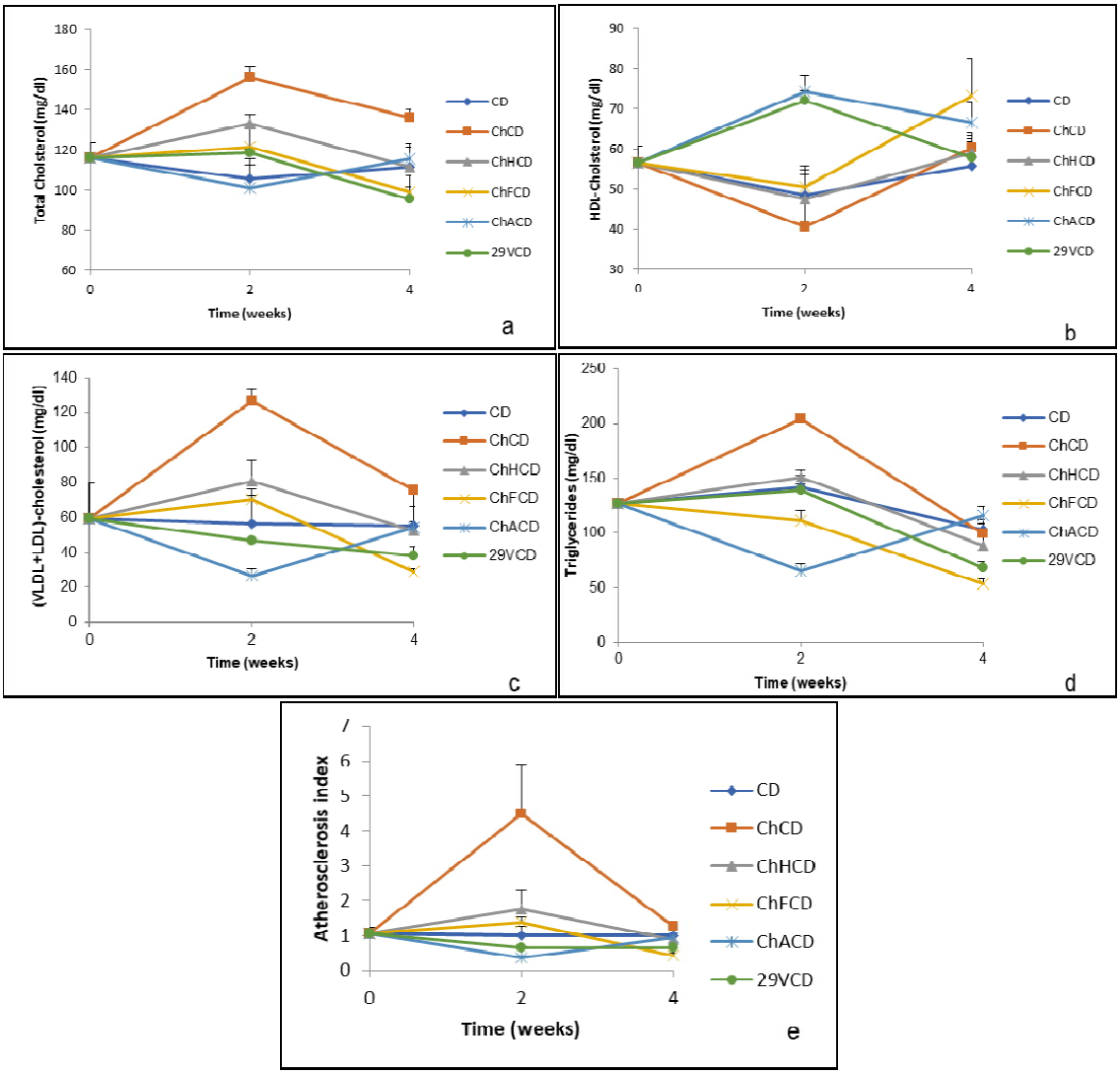

Fig. 2. Lipid profiles during the experimental period

CD : Rats fed Control diet
ChCD : Rats fed pure cholesterol solution (0.04 g/mL) + Control diet
ChHCD : Rats fed pure cholesterol solution (0.04 g/mL) + 0.5 mL of honey + Control diet
ChFCD : Rats fed pure cholesterol solution (0.04 g/mL) + 0.5 mL of probiotic formulation + Control diet
ChACD : Rats fed pure cholesterol solution (0.04 g/mL) + atorvastatine (10 mg/Kg)
29VCD : Rats fed 0.5 mL of Lbp 29V (approximately 10^8 UFC/mL) + Control diet

with pasteurized honey. This would be explained by the fact that the probiotic strain *L. plantarum* 29V although being conveyed by pasteurized honey contributes to the reduction of these parameters because honey would stimulate the growth *in vivo* of lactic bacteria among which *Lactobacillus plantarum* [24]. These results are in agreement with those of Sieladie [8] which showed that this strain administrated alone to rats was able to reduce the total serum cholesterol, (VLDL+LDL)-cholesterol and triglycerides levels. These findings are in agreement with previous reports in rabbits [25], in rats, in mice and humans [26,27,28,23,29]. Conversely, HDL-cholesterol level increased, and the atherosclerosis index was significantly lowered. These results agree with several others and confirmed the moderate action of certain lactic acid bacteria on the HDL-cholesterol [21, 30].

LAB may alter total serum cholesterol by three proposed mechanisms: (a) directly binding, absorbing cholesterol into the cell and assimilation before cholesterol can be absorbed into the body [31,32]; (b) deconjugating bile acids and produce free bile acids, which are more likely to be excreted from the body and drain the cholesterol pool as more bile acids are synthesized [33]; and (c) inhibiting HMG-CoA reductase by some metabolites of lactic acid bacteria like propionic acid [34]. *L plantarum* 29V would reduce the serum cholesterol level through the first two mechanisms: cholesterol assimilation and deconjugating bile acids [7]. In their studies, Bemmo et al. [33] demonstrated that *Lactobacillus plantarum* GLP51 possessed *bsh1* gene encoding for BSH enzyme responsible for the deconjugation of bile salt.

BSH activity may compromise normal lipid digestion and the absorption of fatty acids and monoglycerides could be impaired. Lowering of serum cholesterol-LDL by the lactic bacteria could be related to the reduction of the apolipoprotein B-100 synthesis (proteinic part of the LDL) in the liver and intestine, or the reduction of the transfer of cholesterol esters from HDL to LDL [35]. The increasing cholesterol-HDL level would be due to the synthesis of HDL apolipoprotein called apo A-I, which is the portion of HDL being fixed on the specific HDL receptors [36]. Atherosclerosis index depends on the distribution between the HDL-cholesterol and the (VLDL + LDL)-cholesterol in the blood. Lower is the atherosclerosis index (VLDL + LDL)/HDL), less is the atherosclerosis risk [37].

4. CONCLUSION

The results of this study show that the probiotic strain *L. plantarum* 29V can survive in honey for 28 days and continue to exert its hypocholesterolemic effects after ingestion. Thus, honey could be used as a food matrix to carry the probiotic strain *L. plantarum* 29V very well through the gastro-intestinal tract. This formulation made of pasteurized honey and *L. plantarum* 29V may be used to treat or prevent hypercholesterolemia if these effects are confirmed in Human beings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Huff T, Boyd B, Jialal I. Physiology, cholesterol. [Updated 2021 Mar 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available:https://www.ncbi.nlm.nih.gov/books/NBK470561/
2. Eyup A, Ahmet D, Didar EA. Role of Cholesterol as a Risk Factor in Cardiovascular Diseases. License IntechOpen, Chapter 6. 2018;95-107. Available:http://dx.doi.org/10.5772/intechopen.76357
3. Cardiovascular diseases (CVDs) (who.int). https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). 17 May 2017;6.
4. WHO. Cardiovascular Disease; Fact sheet N°317, Geneva, Switzerland, September; 2009. Available:http://www.who.int/mediacentre/factsheets/fs317/en/print.html (Accessed on 19 May 2010).
5. Zodda D, Giammona R, Schifilliti, S. Treatment strategy for dyslipidaemia in cardiovascular disease prevention: focus on old and new drugs. Pharmacy, 2018;6(10):1-16.
6. Hernández-Gómez JG, López-Bonilla A, Trejo-Tapia G, Ávila-Reyes SV, Jiménez-Aparicio AR, Hernández-Sánchez H. In vitro bile salt hydrolase (BSH) Activity

screening of different probiotic microorganisms. Foods. 2021;10:674. Available:https://doi.org/10.3390/foods10030674
7. Sieladie DV, Zambou NF, Kaktcham PM, Cresci A, Fonteh F. Probiotic properties of lactobacilli strains isolated from raw cow milk in the western highlands of Cameroon. Innovative Romanian Food Biotechnology. 2011;9:12-28.
8. Sieladie DV. Potentiel probiotique des lactobacilles isolés du lait de vache dans les hautes terres de l'ouest du Cameroun. Thèse de Doctorat/PhD. Université de Dschang, Faculté des sciences, Département de Biochimie. 2012;207.
9. Bemmo KUL, Sahoo M, Jayabalan R, Zambou NF. Honey, probiotics and prebiotics: review. Res J Pharm Biol Chem Sci. 2016;7:2428–2438.
10. IUPAC (International Union of Pure and Applied Chemistry). Méthode d'analyse des matières grasses. 6e ed. International digest of health legislation. 1979;46(2):241p.
11. Association of Official Analytical Chemists. In: Helrich K (ed) Official methods of analysis, 15th ed. Arlington, VA USA. 1990;10251026–10331034.
12. Bogdanov S, Martin P, Lüllmann C. Harmonised methods of the European Honey Commission. Apidologie. 1997;29(6):513-524.
13. Telefo PB. Contribution à l'étude des plantes médecinales du Cameroun : Influence de l'extrait aqueux du mélange de feuilles d'*Aloe buettneri* A. Berger (Liliacées), *Dicliptera verticillata* GJH Amshoff (Acanthacées), *Hibiscus macranthus* Hochst ex A-rich (Malvacées), *Justicia Insularis* T. Anders (Acanthacées), sur certains paramètres biochimiques et physiologiques de la reproduction chez la rate. Thèse de Doctorat 3e cycle en Biochimie, Université de Yaoundé I, 1998;154.
14. Trinder P. Determination of total cholesterol, high density lipoprotein cholesterol and riglycerides by enzymatic colourimetric method. Journal of Clinical Pathology. 1969;22158-163.
15. Bemmo KUL, Zambou NF, Rui-Yan W, Zhu T, Yin L. Viability and stress response of putative probiotic *Lactobacillus plantarum* strains in honey environment. Probiotics and Antimicrobial Proteins; 2017a. DOI 10.1007/s12602-017-9358-7.
16. Zambou NF, Moumbe FG, Kaktcham PM. Antimicrobial activity of probiotic strain *Lactobacillus Plantarum* isolated from "Sha'a" and assessment of its viability in local honey. Journal of Microbiology, Biotechnology and Food Sciences. 2013a;3:226-231.
17. Bemmo Kamdem Ulrich Landry, Sahoo Moumita, Jayabalan Rasu, Zambou Ngoufack François. honey, probiotics and prebiotics. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016;7:2428-2438.
18. Jan MS, Mohd NMS, Norrakiah AS. Fructooligosaccharides in honey and effects of honey on growth of *Bifidobacterium longum* BB 536. International Food Research Journal. 2010;(17):557-561.
19. De Man JC, Rogosa M, Sharpe ME. A medium for the cultivation of lactobacilli. Applied Bacteriology. 1960;23:130-135.
20. Probstfield JL, Rifkind BM. The lipid research clinics coronary primary prevention trial: Design, results and implications. Eur. J. Clin. Pharmacol. 1991;40:69-75.
21. Kumar R, Grover S, Batish VK. Hypocholesterolaemic effect of dietary inclusion of two putative probiotic bile salt hydrolase-producing Lactobacillus plantarum strains in Sprague–Dawley rats. British Journal of Nutrition. 2011;105:561–573.
22. Liong MT, Shah NP. Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol co-precipitation ability of lactobacilli strains. International Dairy Journal. 2006;15(4):391–398.
23. Wang Y, Xu N, Xi A, Ahmed Z, Zhang B, Bai X. Effects of *Lactobacillus plantarum* MA2 isolated from Tibet kefir on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. Applied Microbiology and Biotechnology. 2009: 84(2):341-347.
24. Shamala TR, Shri JY, Saibaba P. Stimulatory effect of honey on multiplication of lactic acid bacteria under *in vitro* and *in vivo* conditions. Letters in Applied Microbiology. 2000;(30):453-455.
25. Zambou NF, Kaktcham PM, Fonteh AF, Guetiya WR, Sieladie DV. Effects of Inclusion of Two Probiotic Strains Isolated From "Sha'a", a Maize-Based Traditionally Fermented Beverage on Lipid Metabolism

of Rabbits fed a Cholesterol-Enriched Diet. International Journal of Animal and Veterinary Advances. 2013b;5(2):87-97.

26. Usman HA. Effect of administration of *Lactobacillus gasseri* on serum lipids and fecal steroids in hypercholesterolemic rats. J. Dairy Sci. 2000;83:1705-1711.
27. Bertazzoni ME, Benini A, Marzotto M, Sbarbati A, Ruzzenente O, Ferrario R, Hendrinks H, Dellaglio F. Assessment of novel probiotic *Lactobacillus casei* strains for the production of functional dairy foods. Int. Dairy J. 2004;14:723-736.
28. Suman K, Vibha, Sinha PR. Antioxydative and hypocholesterolemic effect of *Lactobacillus casei* ssp *casei* (Biodefensive properties of lactobacilli). Indian J. Med. Sci. 2006;60(9):360-370.
29. Mahrous H, Shaalan UF, Ibrahim AM. The role of some probiotic lactic acid bacteria in the reduction of cholesterol on mice. International Research Journal of Microbiology. 2011;7:242-248.
30. Kiessling G, Schneider J, Jahreis G. Long-Term Consumption of Fermented Dairy Products over 6 Months Increases HDL Cholesterol. European Journal of Clinical Nutrition. 2002;56:843-849.
31. Gilliland S.E, Nelson CR, Maxwell CV. Assimilation of cholesterol by *Lactobacillus acidophilus.* Applied and Environmental Microbiology. 1985; (49):377-381.
32. Noh DO, Kim SH, Gilliland SE. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. J. Dairy Sci. 1997;80:3107-3113.
33. Bemmo KUL, Kaktcham PM, Momo KCH, Foko KEM, Zambou NF, Wang RY, Zhu T, Yin L. Bile salt hydrolase and antimicrobial activities of three bile resistant probiotics *Lactobacillus plantarum* strains isolated from Cameroonian artisanal fermented milk. Journal of Microbiology and Biotechnology Research. 2017b;7(5):21-30.
34. Fukushima M, Nakano M. Effects of a mixture of organisms, *Lactobacillus acidophillus* or *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat- and cholesterol- enriched diet. British Journal of Nutrition. 1996;(76):857-867.
35. Suzuki Y, Kaizu H, Yamauchi Y. Effect of cultured milk on serum cholesterol concentrations in rats which fed high-cholesterol diets. Animal Science Technology. 1991;62:565-571.
36. Robert KM, Daryl KG, Peter AM. Harper's Biochemistry. Lange Medical Publications. New York USA. 1999;160-295.
37. El-Gengaihi SE, Salem A, Bashandi SA, Ibrahim NA, Abdel-Hamid SR. Hypolipidemic effect of some vegetable oils in rats. Food Agriculture and Environment. 2004;(2):88-93.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67117